REMARKS

Claims 24-26, 28-30 and 32-40 are currently pending in the instant application. No amendments have been made herein to the specification or to the pending claims.

Claim Rejections — 35 U.S.C. § 103

Claims 24-26, 28-30, and 32-40 have been rejected under 35 U.S.C. § 103(a), as obvious in view of U.S. Patent No. 5,968,546 to Baur *et al.* ("Baur"), combined with Lenoir *et al.*, 130 DEV. BIOL. 610 (1988) ("Lenoir") and or Lenoir-Viale *et al.*, 285 ARCH. DERMATOL. RES. 197 (1993) ("Lenoir-Viale").

The Examiner has previously acknowledged that Baur requires further dissection of the hair follicle prior to culturing. (*See* October 23, 2001 Office Action at pages 2-3). However, the Examiner now asserts that Lenoir teaches that intact hair follicles can be used to obtain outer root sheath cells. (*See* Final Office Action, at page 3). According to the Examiner, Figure 2A and its accompanying description in Lenoir demonstrate culturing an intact hair follicle to produce outer root sheath cells, and therefore "provide[d] the reasonable expectation of success that the dissected follicle used in the primary culture of Baur could be replaced with an intact follicle...[while still allowing investigators to] obtain the outer root sheath cells needed for the organotypic culture" described in later steps. (*See* Final Office Action, at page 4). Thus, the Examiner contends that it would have been obvious to one of ordinary skill in the art at the time the instant invention was made to culture the intact hair follicle immediately after plucking (*e.g.*, without the further dissection required by Baur). Therefore, according to the Examiner, the pending claims are obvious over the combined teachings of Baur, Lenoir and or Lenoir-Viale. (*See* Final Office Action at page 5-6). Applicants traverse.

The pending claims are directed to methods for the selection of keratinocyte precursor cells from the outer root sheath of hair, such that the keratinocyte precursor cells can be subsequently used in a composition for healing a skin defect. The methods recited by claim 24 and its dependent claims (including claims 25-26, 28-30 and 32-40) include the steps of (i) plucking an anagenic hair; (ii) primary-culturing the keratinocyte precursor cells by adhering the anagenic hair, *in toto*, to a microporous membrane having growth-arrested limited feeder cells on its undersurface, wherein the primary culture medium contains human serum in a concentration

less than 5%; (iii) organotypically-culturing the outer root sheath cells harvested from the primary cultures by inoculating a microporous membrane also having growth-arrested limited feeder cells on its undersurface, wherein the organotypic culture medium contains human serum in a concentration less than 5% and the keratinocyte precursor cells are seeded at a density of between 3×10^4 cells cm^2 and 1×10^5 cells cm^2; (iv) generating an epidermal or complex skin equivalent for subsequent use as a graft insert; and (v) contacting the epidermal or skin equivalent with a skin defect and immobilizing the equivalent at the site of contact.

Thus, the methods recited by the pending claims culture intact, plucked hair follicles to generate epidermal or skin equivalents for use in healing skin defects. However, Applicants contend that Baur, Lenoir, and Lenoir-Viale, alone or in combination, fail to disclose or suggest culturing an intact hair follicle.

As described above, the Examiner has previously acknowledged that Baur requires further dissection of the hair follicle prior to culturing. (*See* October 23, 2001 Office Action at pages 2-3). Lenoir fails to remedy the deficiencies in the teachings of Baur. Contrary to the Examiner's contention, Applicants believe that Figure 2A and the accompanying description in Lenoir illustrate the detection of the outgrowth of "narrow fringes" of epidermal sheath from an intact hair follicle cultured on a plastic culture dish only in order to determine whether plucked follicles are anagenic, and, therefore, are capable of producing viable epithelial cells. (*See* Lenoir, at 612, second column). This method of selecting anagenic hair follicles disclosed in Lenoir is one example of a variety of methods that can be used by the skilled artisan to determine whether plucked hair follicles are capable of producing viable cells. For example, Baur teaches the visual selection of anagenic hair follicles using a dissecting microscope. (*See* Baur, at col. 4, lines 22-24).

Thus, Applicants contend that the passages cited by the Examiner illustrate a method of selecting anagenic hair follicles, rather than a method of culturing intact, plucked hair follicles to generate a dermal equivalent. In fact, the Lenoir method for culturing the anagenic hair follicles is described further on the same paragraph:

> As an attempt to grow these epithelial cells, hair follicles were *cut into pieces*, and the upper and lower halves were implanted into dermal equivalents and cultured for 5 days under immersion *(see Materials and Methods)*. (Lenoir, page 612, second column, lines 20-25, emphasis added).

Thus, this passage explicitly refers the reader back to the Material and Methods section of Lenoir, which describes, at page 661, first column, lines 14-19, that "the bulbs were removed with scissors, since their soft end would hamper the implantation of the explant into the collagen gel." Similarly, the Materials and Methods section of Lenoir also teaches that the hair follicles were cut into pieces "in order to reduce the size of the explant to ensure that it could keep its upright position." (Lenoir, at page 611, column 1). Accordingly, the Lenoir methods do not use *intact* hair follicles to create epidermal equivalents. Rather, the methods disclosed by Lenoir require further dissection of a plucked hair follicle by removing follicle bulbs with scissors and cutting the follicles into at least two pieces prior to implanting the follicles vertically into a dermal equivalent made of collagen gel. (*See* Lenoir, at 611, column 1). Thus, the stratified epithelium similar to epidermis observed in Lenoir was generated from a dissected hair follicle.

Therefore, contrary to the Examiner's assertion, further dissection of a plucked hair follicle is not simply a matter of convenience for a researcher practicing the Lenoir method of creating dermal equivalents. Rather, further dissection is a necessary step in the Lenoir method. Prior to Applicants' invention, further dissection of the hair follicle to remove the bulb and infundibular parts was the standard method of culturing outer root sheath cells from plucked hair follicles. (*See e.g.*, specification at page 3, lines 12-15; April, 2002 Limat Declaration ¶ 5). Applicants were the first to discover that dissection of the hair follicle after plucking was not necessary. (*See e.g.*, specification at page 2, lines 16-22; April, 2002 Limat Declaration ¶ 5). Accordingly, Lenoir fails to remedy the deficiencies in the teachings of Baur, and, therefore, the combination of Lenoir and Baur does not render the pending claims obvious.

Moreover, the addition of Lenoir-Viale fails to remedy the deficiencies in the teachings of Lenoir and Baur, either alone or in combination. The methods of culturing anagenic hair follicles to create dermal equivalents disclosed by Lenoir-Viale require explantation of a plucked hair follicle onto a substrate of human dead de-epidermized dermis (DED). In addition, Lenoir-Viale teaches away from using other types of matrices or substrates in the disclosed dermal equivalents. For example, Lenoir-Viale teaches, at page 197, column 2, that a DED substrate is more resistant to mechanical stresses and less prone to collagenolysis than a collagen-based matrix. Accordingly, Applicants contend that one of ordinary skill in the art would not have been motivated to modify the Lenoir-Viale dermal equivalents to include a non-DED substrate,

such as, for example, the collagen gel matrix described by Lenoir, or the microporous membranes carrying human fibroblast feeder cells on their underside described by Baur. Thus, for these reasons, the combined teachings of Baur, Lenoir and or Lenoir-Viale fail to render the pending claims obvious. Therefore, this rejection should be withdrawn.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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